REMARKS

Claims Rejections – 35 USC § 112

Claims 1-11 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The Examiner asserts that the claims are generally narrative and indefinite, failing to conform with current U.S. practice. He further comments that the claims are replete with grammatical and idiomatic errors.

The Examiner notes that claim 1 has improper antecedent basis in reciting, "Reagent for the identification". Language such as "A reagent for identifying" is suggested but not required to obviate this rejection.

The Examiner explains that claim 1 is vague and indefinite in reciting, "in particular" because it is unclear whether the limitation following the phrase is part of the claimed invention. Citing MPEP § 2173.05(d).

The Examiner also notes that claim 1 is non-idiomatic, appears redundant, and therefore renders the claim indefinite in reciting, "characterized in that it comprises". The Examiner kindly suggests: "Reagent for identifying..., comprising".

The Examiner asserts that claim 1, (part 1) is indefinite and recites improper Markush language in reciting, "selected from at least one detergent" because it fails to define a selected Markush group of cell lysing agents to select from. A proper Markush group would recite, "a cell lysing agent selected from the group consisting of agent A, agent B, and agent C" for example.

The Examiner has identified a spelling error in claim 1, (part 1), "lye" should be "lyse".

The Examiner further states, regarding claim 1, (part 1) the phrase "specifically a given type of cell" renders the claim indefinite because the claim includes elements not actually disclosed (those encompassed by "specifically a given type of cell"), thereby rendering the scope of the claims unascertainable.

The Examiner holds that claim 1, (part 2) is non-idiomatic and, therefore, confusing in reciting, "designed".

The Examiner suggests that claim 1, (part 2) lacks antecedent support in reciting, "the intracellular nucleic acids" and "the unlysed remaining cell".

The Examiner asserts that claims 2-11 have improper antecedent basis in reciting, "Reagent according to claim". The Examiner suggests language such as "The reagent according to claim" to obviate this rejection.

The Examiner points out that claim 2 is non-idiomatic, incomprehensible, and therefore confusing in reciting, "characterized in that cell lysing agent comprising...in a concentration capable of lysing erythrocytes". He further suggests language such as, "The reagent according to claim 1, wherein the cell lysing agent comprises at least one ionic or non-ionic detergent in a concentration capable of lysing erythrocytes" to obviate this rejection.

The Examiner asserts that claim 2 is indefinite in failing to further limit the subject matter of a previous claim. Specifically, claim 1 from which it depends appears to recite that the "cell lysing agent is selected from at least one detergent"; however, in the instant claim 2, the "cell lysing agent" is recited as "comprising..." which denotes open language being incorporated into the claim.

The Examiner, regarding claim 1 (which we understand to refer to claim 2), asserts that the phrase "and/or" renders the claim indefinite because it is unclear whether the limitation following the phrase are part of the claimed invention.

Claim 3 is non-idiomatic, according to the Examiner, and therefore confusing in reciting, "characterized in that...". He suggests language such as, "The reagent according to claim 1, wherein" to obviate this rejection. The same analogous comment and problem applies to claims 4-11.

The Examiner also asserts that claim 3 is indefinite in reciting improper Markush language in reciting, "the detergent is selected from:". A proper Markush language would recite, "the detergent is selected from the group consisting of...XXX, XXX, and XXX".

The Examiner kindly points out that claim 5 lacks antecedent support in reciting, "the intracellular ribonucleic acid".

The Examiner also suggests that claim 5 is ambiguous in reciting, "and enhancing its fluorescence once it has combined with the latter."

The Examiner asserts that claim 6 is indefinite in reciting improper Markush language in reciting, "stain is selected from:". A proper Markush language would recite, "the stain is selected from the group consisting of...XXX, XXX, and XXX".

The Examiner asserts that claim 7 is vague and indefinite and fails to recite a positive limitation in reciting, "capable of promoting penetration". Specifically, it is also unclear what Applicant intends to encompass in reciting the term, "promoting". A like comment is also applied to claim 8.

The Examiner points out that claim 7 lacks antecedent support in reciting, "the cells to be marked".

Regarding claim 8, according to the Examiner, the phrase "and/or" renders the claim indefinite because it is unclear whether the limitation following the phrase are part of the claimed invention.

According to the Examiner, regarding claim 10, the phrase "and/or" renders the claim indefinite because it is unclear whether the limitation following the phrase are part of the claimed invention.

The Examiner suggests that claim 10 is indefinite in reciting improper Markush language in reciting, "aldehyde selected from:". A proper Markush language would recite, "aldehyde is selected from the group consisting of...XXX, XXX, and XXX".

The Examiner also suggests that claim 11 is indefinite in reciting improper Markush language in reciting, "at least one compound selected from:". A proper Markush language would recite, "at least one compound selected from the group consisting of...XXX, XXX, and XXX".

Claims Rejections – 35 USC § 102(b)

Claims 1-11 are rejected under 35 U.S.C. 102(b) as being anticipated by Sakata (US 5,496,734).

The Examiner asserts that Sakata discloses a reagent for identifying, counting, and classifying blood cells. The reagent comprises a lysing agent or cationic detergent (surfactant) such as quaternary ammonium salts at a concentration that lyses erythrocytes and a fluorescent stain (labeling substance) that can permeate through damaged cell membrane for incorporation with a labeling of intracellular nucleic acids of unlysed cells (leucocytes) (see column 6, line 64 to column 7, lines 1-67 and column 8, lines 1-12). According to Sakata, the Examiner continues, nonionic surfactant may also be added to the reagent to control the effects of the ionic surfactant toward cell membrane (see column 9, line 14 to column 10, line 4). Sakata provides that the surfactant may function by removing part of substances which constitute cell membrane, i.e. ionophore; thus yielding pores in the cell membrane to allow passage of substances such as stain into the cell (see column 10, line 63 to column 11, line 8). Stains used with the reagent include Thiazole Orange, Acridine Orange, ethidium bromide, and propidium bromide (see column 8, lines 18-29). Sakata teaches that alcohol can be added as fixing agent to minimize loss of cytoplasms and granules and to optimize degree of damage of the cell membranes. According to Sakata, formaldehyde and gluteraldehyde are also used as fixing agents (see column 10, lines 10-39 and column 2, lines 13-19). The reagent further includes anticoagulant and buffer (see column 8, line 64 to column 9, line 13).

Claims Rejections – 35 USC § 102(e)

Claim 1-7 and 9-11 are rejected under 35 U.S.C. 102(e) as being anticipated by Sakata (US 5,496,734).

The Examiner may have intended a rejection over Deka et al. (US Patent No. 6,261,035). We respond both to Sakata et al. and Deka et al. as references.

The Examiner states that Deka et al. discloses a reagent for identifying, counting, and classifying blood cells using flow cytometry. The reagent comprises a lysing agent or non-ionic detergent and a fluorescent dye. The non-ionic detergent may be polyoxyethylene ethers and sorbitans (Tween X) (see column 4, lines 46-59). Stains used with the reagent include Acridine Orange and TO-PRO-3 (see column 6, lines 9-22). According to Deka et al., cell fixation is used to transport or penetrate fluorescent stain into cells through cell membrane to stain RNA (see column 5, line 14 to column 10, line 4). Deka et al. teach using formaldehyde as fixing agent; hence, a cell penetration agent (see column 5, lines 53-66). The reagent further includes buffer fixatives (see column 5, lines 12-19).

Prior art made of record are not relied upon but considered pertinent to the applicant's disclosure:

According to the Examiner Kim et al. (US Patent 5,648,225) disclose a multipurpose reagent having a lysing agent (saponin) and a nuclear stain (ethidium bromide).

The Examiner states that Lefevre et al. (US Patent 5,232,857) disclose a reagent having a lysing reagent (saponin) and a stain (chlorazol black).

Murphy et al. (US Patent 6,562,563), according to the Examiner, discloses compositions for determining interactions of mitochondrial components. Also, that Murphy et al. teaches assessing the ability of agents to modulate apoptosis using ionophores such as valinomycin (see columns 36 and 37).

ARGUMENTS

Claims 1 to 19 are pending in the application. Claims 1-11 are rejected and have been amended upon entry of this Response. Claims 12-18 are withdrawn from consideration. Claim 19 is new. No new matter has been added.

English language abstracts of EP 0343380 and FR2759166 are provided with this Response. Applicant respectfully requests that these references be considered by the Examiner.

The Abstract has been amended to place it into proper format.

The Specification has been amended in accordance with the suggestions of the Examiner.

All rejections that are not overcome by amendment are respectfully traversed for the reasons indicated in this Response.

Claim Rejections 35 U.S.C. § 112

Claims 1-11 have been amended to obviate the rejections under 35 U.S.C. § 112 detailed above.

Claim 1 uses the language "specifically a given type of cell" which the Examiner identified as rendering the scope of the claim unascertainable.

Applicant respectfully notes that the term is explained in the text, for example: "[A] reagent that enables lysis of certain cells, in particular erythrocytes, the fixing of nucleate cells and the staining of the intracellular material to be carried out simultaneously." Page 5, ll. 17-20. As the MPEP points out: "Some latitude in the manner of expression and the aptness of terms should be permitted." MPEP 2173.02. Applicant suggests that the scope of the claim would be clear to one of skill in the art because, at least, biological samples have a limited number of types of cells. For at least these reasons, the scope of claim 1, as amended is easily ascertainable.

Claim 1 has been amended to remove the phrase *"in particular in a blood sample."* While in one embodiment this invention has an application to blood samples, it may also be used for other biological liquids or biological preparations, as outlined in this specification.

In other words, the cell lysing agent lyses a given type of cell (erythrocytes in case of a blood sample) and does not lyse remaining cells which have a nucleus.

Claim Rejection – 35 USC § 102

The Examiner rejects claim 1-11 under 35 USC § 102(b) as being anticipated by Sakata (US 5,496,734).

More particularly, the Examiner considers that Sakata discloses a reagent which comprises *"a lysing agent or cationic detergent (surfactant) such as quaternary ammonium salts at a concentration that lyses erythrocytes and a fluorescent stain (labeling substance) that can permeate through damaged cell membranes for incorporation with and labeling of intracellular nucleic acids of unlysed cells (leucocytes)".*

The Examiner refers to column 6, line 64 to column 7, lines 1-67 and column 8, lines 1-12 of the reference.

He also adds that a *"non-ionic surfactant may also be added to the reagent to control the effects of the ionic surfactant toward cell membrane".*

It is respectfully submitted that Sakata does neither disclose nor suggest the reagent of this invention and the particular functions accomplished thereby.

Although Sakata discloses a reagent comprising a surfactant and a labeling substance, these two components are different from, and do not function in the same way, as in this invention. Thus, Sakata et al.'s invention is directed to a method in which "leukocytes can be selectively classified and counted by treating the whole blood sample without removing erythrocytes and blood platelet cells." Col. 6, ll. 59-63.

By contrast the reagent of the instant invention specifically lyses erythrocytes. Thus the inherent nature of the reagent differs.

Sakata uses at least one surfactant selected from the group consisting of a cationic surfactant and an amphoteric surfactant at a concentration that does not destroy the whole cell membrane of leucocytes but is sufficient to slightly damage a part of the cell membrane so at to

make it permeable (col. 2, line 62-col. 3, line 8 and col. 7, lines 48-55). Thus, the surfactant of Sakata is designed to damage a part of the cell membrane of a leucocyte, whereas in this invention the surfactant (detergent) is designed to lyse erythrocytes (not leucocytes), if the sample is a blood sample.

The Sakata reagent also uses a labeling substance, which is capable of passing through the damaged cell membrane and combining with a component contained in the treated leucocyte.

Consequently, according to Sakata, the action of the labeling substance on the leucocytes is permitted by the action of the surfactant, which slightly damage a part of the cell membrane of the leucocyte to make it permeable.

By contrast, in this invention, the stain is designed to mark the intracellular nucleic acids of unlysed cells, particularly leucoctyes, which have not been damaged by a surfactant. Thus, the two components are inherently different.

Consequently, the respective functions of the surfactant and the labeling substance are not directed to leucocytes in the case of the Sakata reference. Sakata does not seek to lyse the erythrocytes and leave the leucocytes unlysed. Rather, Sakata seeks to damages to cell membrane of the leucocytes.

Sakata also refers to a non-ionic surfactant (col. 9, line 14 and following).

According to Sakata, such a non-ionic surfactant may be added to the aqueous reagent solution of the cationic or amphoteric surfactant and the labeling substance.

Starting from line 50 of page 9, Sakata explains that *"The mechanism of the function of the non—ionic surfactant is not made clear, but is considered to be that the non-ionic surfactant is combined with the surface of the cell to control the acting of the ionic surfactant toward the cell membrane, thereby inhibiting the lyses of cell components with the ionic surfactants"*.

And Sakata adds: *"For example, the use of the ionic surfactant associated with the non-ionic surfactant is preferable when the ionic surfactant has so potent a lysing activity that leucocytes are unnecessarily damaged"*.

From this, we understand that Sakata wishes to control the function of the surfactant in such a way that membranes of the leucocytes are permeable to the dye. By contrast, in this invention, one of the functions of the reagent is precisely to lyse the erythrocytes, where the sample is a blood sample.

Claim Rejections 35 USC § 102

The Examiner rejects claims 1-7 and 9-11 under 35 USC § 102(e) as being anticipated by Deka et al. (US 6,261,035). We respectfully point out the apparent error in referring to Sakata, instead of Deka.

The Applicant respectfully submits that Deka et al. does neither disclose nor suggest the instant reagent.

Although Deka and al. refer to methods and compositions for rapid staining of nucleic acids in whole cells, the reference is not at all relevant.

Deka et al. is directed to the study of reticulocytes, which are a particular type of red cell which have RNA debris but no nucleus.

By contrast, in one embodiment this invention claims a reagent for identifying and counting nucleate cells upon lysis of red cells (including reticulocytes).

Such two type of cells are distinct. Erythrocytes (red cells) have no nucleus. The invention aims to identify and count nucleated cells including leucocytes and erythroblasts.

Deka et al. use a detergent, not to lyse cells, but only to modify the membrane plasticity, and a sphering agent (in order to make the red cells spherical). Consequently, the purpose of

Deka is not to lyse the red cells, but, by contrast, to leave them intact, which is absolutely different from this invention.

All the claims which depend directly or indirectly from claim 1 are allowable upon allowance of claim 1 because they incorporate all the limitations of the independent claim.

Moreover, with reference to claim 8, neither Sakata nor Deka use an ionophore. The term ionophore has specific meaning in the art, a meaning which differs from the Examiner's apparent usage.

New claim 19 is believed allowable because the prior art of record does not disclose the combination of agents claimed, as those terms are understood by one of skill in the art.

In view of the above amendments, applicant believes the pending application is in condition for allowance.

Applicant believes no fee is due with this response. However, if a fee is due, please charge our Deposit Account No. 22-0185, under Order No. 20198-00059-US from which the undersigned is authorized to draw.

Dated: March 22, 2005

Respectfully submitted,

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